RESEARCH ARTICLE

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The Use of Non Dairy Creamer Wastewater as the Growth Medium of *Saccharomyces cerevisiae* for Single-Cell Protein Production

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This Non dairy creamer (NDC) wastewater have a potential as microorganism growth medium. This research had an objective to find out the potential of non dairy creamer industrial wastewater as the growing medium for *Saccharomyches cerevisiae* and protein production within the non dairy creamer wastewater. The research applied a full factorial design, by which treatments composed of multi-factoral combination were used and Multivariate of Anova data analysis. The treatments consisted of P1 (NDC medium concentration 25%), P2 (50%), P3 (75%), and P4 (100%), with the concentration determined for cells of 10^5, 10^6, 10^7, and 10^8. According to the Multivariate of Anova analysis, the observatory opportunities of the medium and cell concentration resulted in p 0.000, or $p < 0.05$. Therefore, H_0 was rejected as interaction between the medium concentration with cell concentration and single-cell production existed. The medium concentration determined at 100%, 75%, 50% and 25% resulted in significantly no difference from the number of cells and the cell dry weight at $p > 0.05$, but significantly difference from the protein content at $p < 0.05$. The highest protein content was obtained by the medium concentration of 75% and cell concentration of 10^6 (cell/ml).

Keywords: *Saccharomyces cerevisiae*, Non Dairy Creamer Industrial Wastewater.

1. INTRODUCTION

There as been a great expectation for vast growing industrial sector nowadays to be able to help achieve people welfare. However, as industries keep growing, they also leave problems in the form of wastes. The industry of non dairy creamer is a food ingredient company, which makes use of coconut oil as raw material. Non dairy creamer exerts wastewater that have caused contamination due to poor management. Furthermore, the wastes have also diminished water quality and bad smells. The smells derive from excessive organic compounds contained within the liquid wastes. Accordingly, there is an urgent need for a good management by making use of wastes to be valuable raw material.

There have been studies on wastes as a microorganism growth medium for single-cell protein production. This method has been believed more affordable. However, no research has been performed on the use of the non dairy creamer for the similar purpose. The use of *Saccharomyces cerevisiae* in processing the non dairy creamer wastewater is expected to give a promising prospected in the single-cell protein production. The single cell protein (SCP) is a dry cell, or biomass, of such microorganism as *khamir,* bacteria, and *ganggang,* which can be used for alternative to fulfill the needs for proteins in the future. It is due to the fact that it contains particular proteins, carbohydrate, fat, mineral and other nutrients necessary by either human or animals.[1–3] The use of the *Saccharomyces cerevisiae* in the processing of the non dairy creamer wastewater is expected to have a promising prospect in the production of single-cell protein.

Laboratory analysis has reported that non dairy creamer wastewater contain organic substances, all with great potential to become a growth medium for microorganisms. Efforts in making use the non dairy creamer wastewater can be performed by altering them as raw materials. In this context, raw materials refer to microorganism fermentation medium developed for producing single cell protein. Therefore, a research on the use of the non dairy creamer wastewater as the growth medium of *Saccharomyces cerevisiae* for the production of single-cell protein is considered necessary.

2. EXPERIMENTAL DETAILS

A experimental research was performed at the Chemical Laboratory of the Faculty of Sciences and Mathematics of the Satya

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Wacana Christian University, Salatiga Research then followed up by analyzing efficiency of non dairy creamer liquid processing for producing *Saccharomyces cerevisiae* based on

(a) length of time of the optimal growth by number of cell and cell dry weight,
(b) produced protein content, and
(c) biosorption rate of *Saccharomyces cerevisiae* during fermentation process of the non dairy creamer wastewater medium.

The research used a full factorial design, in which treatments consisted of multi-factorial combination.

The first stage was characterization of fermentation media of non dairy creamer wastewater. The second stage was optimization according to full factorial design comprising of independent variables concentration of non dairy creamer wastewater and concentration of *Saccharomyces cerevisiae* cells, and dependent variables number of cells, cell dry weight, concentration of produced protein. The third stage was laboratory experiment. It consisted of *YEPD medium processing, inoculum preparation* following,[4] *waste medium characterization for production medium,*; *non dairy creamer wastewater medium preparation* with concentration of 100%, 25%, 50%, and 75%, *S. cerevisiae growth processing in YEPD and non dairy creamer wastewater media* following.[5] The media were also subject to a pH and *Saccharomyces cerevisiae* cell population scalings every 24 hours in seven days; *medium pH scaling* of YEPD medium and of non dairy creamer liquid waste medium took place at 0th, 24th, 48th, 72th, 96th, 120th, and 144th hours using a pH meter; *number of S. cerevisiae cells scaling* by cells/mL each 24 hours following,[6] *S. cerevisiae growth processing at optimum waste concentration* as determined by vary concentration of the *S. cerevisiae. S. cerevisiae cells powder preparation* following.[5] Following this process, the cells were crushed to become powder. Using Kjeldahl micro method, the powder were subject to protein content and dry weight scalings; and *S. cerevisiae bisorption potential calculation* by analyzing carbohydrate (glucose), nitrogen and fat (glicerol) contents in the medium before and after fermented condition.

3. RESULTS AND DISCUSSION

Single-cell protein production to be examined was that of variation of substrate concentration and variation of cell concentration to obtain the best growth rate of the *Saccharomyces cerevisiae* in the process of fermentation of the non dairy creamer wastewater medium. According to the Multivariate of Anova, the observatory opportunity of the medium and cell concentration was p 0.000, or $p < 0.05$. In other words, interaction between medium concentration with cell concentration and single-cell protein production existed.

Furthermore, Figure 1 (marginal of means) summarizes that concentration of *Saccharomyces cerivisiae* inocculum affected protein production. The highest protein contents were found at medium concentration 75% and cell concentration 10^6 (cells/ml). Following,[7] the inocculum addition with low concentration caused slow fermentation rate, but produced the higher products, because after the cells multiplied, they would gradually convert substrate into products.

That in making ethanol, too high *Saccharomyces* (yeast) inocculation may weaken the process more immediately and decrease cell viability after the growth phase. The growth and metabolism

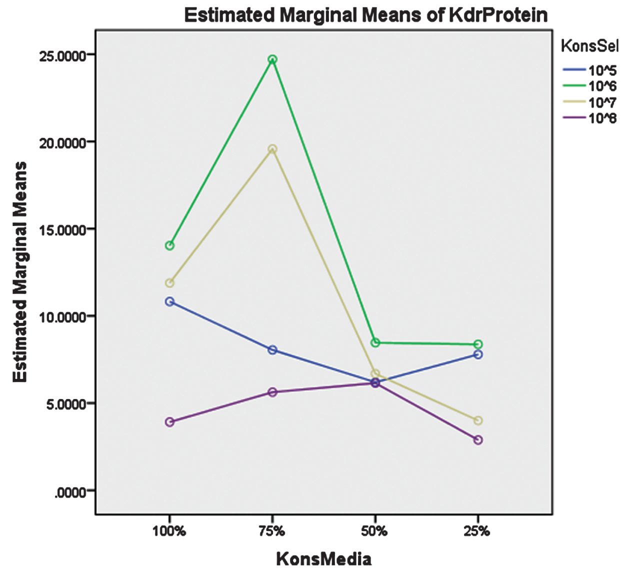

Fig. 1. Marginal of means of protein contents in vary media and cell concentration.

in the cells with high population was unexpected because it disturbed nutrition access, space and intercellular interaction.[8]

The media concentration 100% resulted in the lower protein contents than that of media concentration 75%. Too high substrate concentration reduces dissolved oxygen. However, limited storage of oygen is still necessary for the fermentation by *Saccharomyces cerevisiae* to survive in high cel concentration.[9,10] Oxygen were needed to produce ATP in glycolysis and in oxidative phosporization. The final process is the most notable reaction to produce ATP. If there is not oxygen (anaerobic), NADH within mitochondria may not be re-oxidated, and therefore the formation of the ATP, citric acid cycle as well as other nutrition decomposition will also be terminated. As a static energy substrate there is only glucose, which decomposed into piruvate through a glucolysis, a process that resulted in two ATP molecules.

Medium is a material, which consists of nutritious substances necessary by microorganisms for their growth.[11] Concentration medium 75% contains carbohydrate, fat, and protein preferrable for the microorganism growth (*Saccharomyces cerevisiae*), which performs a series of metabolic process. In the metabolism carbohydrate is converted into glucose and, in turn, other compounds such as organic acid, aldehyde or alcohol. Whereas, fat metabolism converts protein into amino acid.

Glucose as the main source of carbon is absorbed through a process of active transfer, which in turn is subject to metabolism to produce energy and to synthesize cell-developing material and metabolic synthesis. Whereas nitrogen source within the fermentation medium is used for synthesizing proteins within cel.[12,13]

Glicerol is a lipid derivate that can be dissolved in water. The presence of the glicerol in the medium is important because it can protect antimicrobial activities by means of improving stability of the genuine protein structures of the microbe, preventing proteins from thermal and aggregation process. In addition, glicerol can also be used as energy source.[14]

Nitrogen is the main component of the protein and nucleic acid, contributing to 10% of the total cell dry weight. Provided that all proteins and nucleic acids contain nitrogen, some of

the nitrogens are needed for growth, even nitrogenous components can be determining factors that restrict activities and growth of *Saccharomyces cerevisiae*.[15] Growth is an event of improving all cellular-chemical major elements. It is a process, which needs replication of all structure, organel, and cellular protoplasm, with the presence of nutrient and surrounding environment. In microbial growth, all essential substantials have to be available for protoplasm synthesis and preservation, using proper energy source and environmental condition. Cell concentration determined at 10^6 (cell/ml) in the media showed the best value of the proteins to be produced. Following,[6] number of cells ($\times 10^6$ cell/ml) of *Saccharomyces cerevisiae* held in tahu-coconut oil waste medium for 96 hours resulted in an optimal single-cell protein.

According to the results of research,[7] which states that the addition of inoculum with a low concentration resulted in the fermentation rate to be slow, but it can produce higher because after multiplying cells, the cells will convert the substrate into the product gently. Following,[8] in ethanol production, inocculation of too high concentrated *Saccharomyces* (yeast) may weaken the process faster as well as lower cellular viability after growth phase. Growth and metabolic condition in cells with high population rate is unexpected because it can distort nutrition acess, space availability, and intercellular interaction.

Following,[16] writes that *Saccharomyces cells* consist of lipoprotein that contains enzymes necessary for the synthesis of part of cellular wall components. Enzymes available in the *Saccharomyces cerevisiae* include protease, carboxypeptidase, amminopeptidase, and inverase. These enzymes allow *Saccharomyces cerevisiae* to make use of waste medium as their growth medium.

The total protein contents, based on substrate concentration, resulted in the highest result in concentration medium 75%. It was made possible because the nutrition contents within the concentration 75% were still relatively adequate for growth, affecting the proteins to be produced. According to the results of research[17] suggests that nutrients that contain glucose will supply energy for the metabolism process of *Saccharomyces cerevisiae,* whereas the making of non dairy creamer products also involved glucose.

In addition to glucose, the laboratory analysis has found that non dairy creamer liquid waste medium also contains glicerol, which can be used by *Saccharomyces cerevisiae* when the glucose contents are less available in the medium. Glicerol can be used as energy source.[14]

According to Ref. [18], the better nutrients within the growing substrate, the faster growth rate the cells will be and it may increase the cell protein contents. Furthermore, cell protein contents are affected by reproduction time. Too short reproduction time produces less protein becuse bioconversion of the substrate components has not been optimal. Conversely, too long reproduction time means that there is less protein due to autobiodegradation to fulfill energy needs relative to nutrient availability within the medium, which is less and less adequate.

The protein contents of 35.88 mg/l were obtained on the concentration of medium 75% when the fermentation had lasted 24 hours. The highest exponential phase was obtained on cell concentration 10^6. During the exponential phase the cells grew fastly so that their masses and numbers increased exponentially as the time went by. When the masses grew fast, the cell components grew as the proteins developed.

4. CONCLUSION AND RECOMMENDATION

Variation of substrate concentration and cell concentration of *Saccharomyces cerevisiae* affect proteins they produce. The highest rate of protein contents were found on concentration of media 75% and concentration of cell 10^6 (cell/ml).

A further analysis is necessary to find out the effect of addition of other nutrition, such as minerals, into the non dairy creamer, to improve potential as the growth medium for the single-cell protein.

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